Micropropagation Techniques for Rare Plant

Subjects: Plant Sciences

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The current investigation aimed to present an overview of the conservation of biological diversity of rare and endangered plant species. Methods of biodiversity conservation as well as several overview recommendations for the preservation of various rare species have been considered. An overview of the taxa included in the red book has been presented on the example of the Russian Federation. Global and local codes and classifiers of plant rarity were also presented. Future prospects for the conservation of biological diversity and the creation and development of bioresource collections have been considered.

Keywords: biotechnology,red-list plants,micropropagation,in vitro,plant tissue culture,rare plants

1. Introduction

Nowadays, one of the most pressing issues in biology is the preservation of the genetic diversity of living organisms. Special attention is paid to the conservation of the gene pool of many plant species since the most often the price-forming organisms in nature for breeder-preferred traits, as well as consumer-preferred traits [1][2]. The important preserving gene pool is not only for vital agriculture, but also for rare and endangered plants, which often have medicinal, decorative, forage, and other properties. Also, rare plants are important components of vegetation in a particular region. Their disappearance can lead to the destruction of the essence of the biological flora of plant communities [3].

Various plant species in Europe, Asia, America, and in Russian Federation; particularly in the Rostov region of Russia are currently under threat of extinction and have one or another status of rarity. The reasons for the rarity of some plants in this area are mostly the anthropogenic load in the form of plowing land, grazing, construction, and the low competitiveness of the plant species in phytocenoses. Other influencing factors for the propagation of these rare plant species are low seed germination or vegetative reproduction, relict species, torn areas, harsh climatic conditions, eaten by animals and birds, etc. [2][4][5]. Several methods have been adopted to preserve the plant gene pool, for example, (a) collection of closed and open ground-based botanical gardens and nurseries, (b) creation of reserves, and other specially protected natural areas (SPNA), and (c) modern biotechnological tools to create seed banks, cells, tissues, and pollen storage, and organization of banks of plant genetic material, i.e., micropropagation. The modern biotechnological tools have been evolved as the fastest, and most efficient strategies to preserve the plant's gene pool [2][6][7].

Today, the in vitro culture method is widely used to solve the problems of preservation and restoration of the gene pool of rare and endangered plant species. In addition, this method is able to provide the material in a larger amount for plant breeding programs at specific sites. The endemic habitats of rare and endangered species are often hard to reach for various specific purposes. It is associated, first, with the formation of callus, suspension, meristematic cultures, cultures of ovules, anthers and pollen, cryosurgery of tissues; secondly, the development of technologies for reproduction with the prospect of their further dedifferentiation and redifferentiation. Creating plant in vitro collections can be considered as a protection form of plants of natural flora, and an effective method of their ex situ biodiversity preservation, which is part of the overall strategy for plant protection [6][7][8][9].

2. Emerging and Updated Micropropagation Techniques for Rare Plant Species

New methods in emerging and updated micropropagation of rare and endangered species of plants, standard technologies for selecting cultivation conditions are most often discussed. For rare plant species that have not been previously regenerated in vitro, it is very important to optimize the most effective combinations of macro- and microelements, vitamins, amino acids, growth regulators, as well as, sometimes, antibiotics and substances that bind phenolic compounds. It is necessary to take into account the chemical composition of the soil of plant communities where a rare plant grows, which is necessary for introduction into culture [10].

However, today, there are some new techniques and technologies that are useful for in vitro cultivation and which are not only species-specific but also for use with many species of threatened plants that are close in systematic or ecological terms. Recently a robust regeneration method for a crop wild legume and rare plant species “*Cicer microphyllum*” has been demonstrated to conserve the germplasm and to ensure the availability of germplasm for breeding programs [11]. This wild legume has been proved as a natural repository of valuable traits for crop improvement programs [12][13][14][15]. However, this species has endemic habitats in cold deserts of Himalayan mountain regions and diminishing very fast due to overgrazing by animals, and seeds were being eaten by birds. The method optimized for this species could help for large-scale propagation of disease-free plants in a containment facility for breeders to use in a crop improvement program, and may be useful for other rare wild legume species.

Some endangered plants are grown in bioreactor systems. Many studies indicate that growing plant tissues and organs in liquid and semiliquid media in a bioreactor is faster and more efficient than using solid media due to maximum supply of nutrients and hormones to explants, better contact with medium and aeration system for maximum growth for scaling up purposes [16]. Studies using bioreactor systems have been performed for many plant families with a large number of threatened species: *Orchidaceae* Juss. [17], *Araceae* Juss. [18], *Plantaginaceae* Juss. [16], *Rosaceae* Juss. [19], *Asteraceae* Bercht. and J. Presl [20], *Arecaceae* Bercht. and J. Presl, nom. cons. [21], *Moraceae* Gaudich. [22]. Due to their technological properties, bioreactors contribute not only to a fast and high-quality micropropagation process but also to the ability to quickly obtain substances useful for medicine from endangered plant species, especially from roots [17].

However, not all plant species are easily regenerated by in vitro culture. This is especially true for many rare plant species. Cells, tissues, and organs of some rare plant species are difficult to cultivate at one or more stages of micropropagation. These plants are called “recalcitrant” [23]. For “recalcitrant” plants, it is necessary to search for suitable explants, nutrient media, and adaptation methods, which is difficult with tree species, aquatic plants and plants of the family *Orchidaceae* Juss.

When cultivating aquatic plants, biotechnology face a high degree of contamination of plant material, as well as low seed germination [24]. To avoid in vitro contamination of aquatic plants, new types of explants must be sought. A striking example is the representatives of the family *Nymphaeaceae* Salisb family. The species *Nymphaea* ‘Daubeniana’ has special epiphyllous shoots that can be used for micropropagation [25]. However, this is the only type of water lilies that has this feature. For many other water lilies, it is recommended to use unfertilized bud ovaries as explants, or seeds, if any [24]. In the case of orchids, difficulties arise when using seed material devoid of endosperm [26], as well as when adapting them ex situ, since many orchids are highly specialized species adapted to specific habitats. Acclimatization of many orchids, such as the ghost orchid (*Dendrophylax lindenii*), is a multi-stage and long-term process [24].

It is important to note the problem of the symbiosis of orchids with mycorrhizal fungi. Due to the almost complete lack of nutrients in the seeds of orchids, these plants are forced to enter into a symbiotic relationship with some mycorrhizal fungi. Without mycorrhizal fungi, even with micropropagation, the cultivation of orchids can become almost impossible. Therefore, in the case of orchids, introducing fungal cultures into in vitro cultures specific to each particular type of orchid is actively practiced. This technology is particularly important for the acclimatization of orchids to indoor and outdoor conditions [27].

The basis for rapid growth and obtaining certain organs and tissues at a particular stage of micropropagation is the use of various growth regulators. Among the many commonly used phytohormones, rarer substances sometimes appear in vitro. One of these is triacontanol, which stimulates the formation of chlorophyll and increases the intensity of photosynthesis. The effect of the presented phytohormone is described on such plants as sweet wormwood (*A. annua*) [28], apple (*Apple domestica*) [29], lemongrass (*Cymbopogon flexuosus*) [30], noble dendrobium (*Dendrobium nobile*), etc. [31].

Protoplast culture is sometimes used to preserve and study the genetic component of rare and endangered plants. This provides great convenience when extracting the genome or transcriptome [32]. Recently, some narrowly focused techniques used for micropropagation of plants have been developed. These include in vitro micrografting and facilitates the cultivation of many woody plants [33]. An interesting and important problem is the hyper hydracity of explants. It can interfere with the growth and rooting of regenerating plants. To get rid of waterlogging, various polishing agents are used that differ from the usual hangar [34].

A relatively new technique in the micropropagation of both rare and many other plants is biotization of endophytic microorganisms. This technology is designed to stimulate growth, development, reduce stress, and increase plant immunity in vitro by introducing bacteria and fungi into cultures [35]. New studies have revealed a positive effect of many microorganisms on the growth of the vegetative part of plants, seed maturation, resistance to pathogens, callus growth, and increased tolerance to low temperature [36].

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